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ORIGINAL PAPER

Effect of low-protein diet with supplementing different levels of DL-methionine on production performance of minks in growing-furring period

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Abstract: A study was conducted to evaluate production performance of minks in growing-furring period with supplementing DL-Methionine (Met) in low protein diet. Seventy healthy male minks were randomly divided into five groups of 14 minks each. The minks were fed in five kinds of experiment diets (HP, LP, LP+M1, LP+M2 and LP+M3). The dietary protein levels, expressed as percentage of dry matter (DM), were 32% (high protein, HP) and 24% (low protein, LP). LP was supplemented with Met 0.4% (M1), 0.8% (M2) and 1.2% (M3) DM. From mid of September to December 10, apparent digestibility of CP (crude protein), N intake and urinary N excretion were decreased with declining dietary protein levels ($p < 0.05$) and N retained was the highest in treatment LP+M2. No significant difference was found in total serum protein (TP) and serum urea nitrogen (SUN) among all treatment groups ($p > 0.05$). Skin length of treatment HP and LP+M2 was higher than that of other groups ($p < 0.05$). Body length, skin weight, length of guard hair and under hair were not affected by different dietary protein levels ($p>0.05$). The best performance could be observed in treatment LP+M2. In diet, 24% (DM) protein level with 1.54% Met supplementing was enough for minks during growing-furring period. Dietary protein lowered from 32% to 24% with supplementing Met in diets would result in a 37.9% decrease in urinary N excretion. Furthermore, addition of Met in diets for minks would be beneficial in terms of reducing feed expenses and lessening nitrogen emissions to the environment.

Keywords: minks; low-protein diet; DL-methionine; fur characteristics

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Introduction

Mink (*Mustela vison*) is a carnivorous species with a high protein requirement (NRC 1982; Hansen et al. 1991). Protein is the most expensive dietary nutrient; any reduction in protein level contributes to a saving in production cost and to a reduction in N emissions. Many experiments had proved that supplementing low-protein diets with amino acids may increase the metabolic value of the diet and improve the growth performance of pig and poultry (Easter et al. 1980; Quiniou et al. 1994; Kerr et al. 1995). According to Børsting and Clausen (1996), Met is the first limiting amino acid in mink diets. Amino acid requirements of mink have been the subject of several studies, including studies on the effects of different protein levels and amino acid composition on the growth, the fur quality and the health of mink (Børsting et al. 1996; Dahlman et al. 1996; Glem-Hansen 1990). However, the possible effects of the low-protein dietary with supplementing Met at different levels have not been studied during growing-furring period of minks. Animal farming is of great environmental concerns due to its huge emission of feces and urine, thus technology to reduce the emission is a hot topic; Diet optimization should concern four indexes, such as the emission effects, normal physiology of animals, productivity and cost effectiveness. Our hypothesis is that adjusting crude protein may alter the above four indexes and we take the farmed mink as a model to test the hypothesis.

The objective of the present study was to investigate the effects of low protein levels of dietary with Met supplementation on the performance, N retention, nutrient digestibility, some blood parameters, and fur characteristics of minks.

Materials and methods

Experimental design

The experiment was carried out at the fur farm of the Institute of Special Economic Animals and Plants, Chinese Academy of Agricultural Science, Jilin City, in the period from September 16th to December 10th. Seventy healthy male minks were housed in roofed standard sheds with open sides. The average (±SD) age and weight of animals at start were (126±6) d and (1.95±0.20) kg, respectively. The animals were allotted to five treatment groups containing 14 animals in each. The experiment was preceded to feed animals at one week. The ingredient and composition of experiment diets are listed in Table 1. Chemical compositions of diets are listed in Table 2. Animals were free access to drinking water and were fed twice a day with the experimental diets. All experimental data of animals were performed by the same person. Body weight (BW) of animals was determined every 15 days.

Blood samples were collected from eight males per experimental group at the end of experiment and brought to laboratory quickly and centrifuged for 10 min at 5000 rpm. Then the serum was separated from blood and transferred into Eppendorf centrifuge tubes, and kept at −20°C until analysis.

Table 1. Ingredient composition of the experiment diets (fresh matter) (%)

Treatments	Extrusion corn	Corvina	Poultry offal	Eggs	Jarding pork	Hircine liver	NaCl	Methionine	Premix[1)]	Water	Total
HP	11.00	38.00	5.00	4.00	4.00	5.00	0.50	-	0.50	32.00	100.00
LP	14.00	27.00	5.00	2.00	6.00	4.00	0.50	-	0.50	41.00	100.00
LP+M1	14.00	27.00	5.00	2.00	6.00	4.00	0.50	0.08	0.50	40.70	100.00
LP+M2	14.00	27.00	5.00	2.00	6.00	4.00	0.50	0.16	0.50	40.40	100.00
LP+M3	14.00	27.00	5.00	2.00	6.00	4.00	0.50	0.24	0.50	40.10	100.00

Notes: [1)] The premix provides ingredient composition for per kg diet as follows: Fe (as ferrous sulfate) is 80.0 mg; Cu (as copper sulfate) 20.0 mg; Zn (as zinc sulfate) 50.0 mg; Se (as selenium sulfate) is 0.2 mg; Mn (as manganic sulfate) is 60.0 mg. Vit A is 300 IU; Vit B1 0.15 mg; Vit B2 0.4 mg; Vit B6 is 0.3 mg; folic acid is 0.3 mg; nicotinic acid 1.6 mg; D-pantothenic acid is 1.3 mg. HP is 32%CP (high protein level diet); LP is 24% CP (low protein level diet); LP+M1 is 24% CP+0.3% Met; LP+M2 is 24% CP+0.6% Met; LP+M3 is 24% CP+0.9% Met.

Table 2. The chemical compositions of the experimental diets

Treatments	Dry matter (%)	Ash (%)	Crud protein (%)	Fat (%)	Carbohydrates (%)	ME ($MJ \cdot kg^{-1}$)	Calcium (%)	Phosphorus (%)	Methionine (%)
HP	24.11	8.58	32.64	22.50	36.28	21.48	1.65	0.79	1.08
LP	25.33	6.38	24.93	22.98	45.71	21.88	1.33	0.64	0.72
LP+M1	25.04	6.33	25.28	22.32	46.07	21.74	1.12	0.74	1.19
LP+M2	25.10	6.21	25.79	21.83	46.17	21.66	1.09	0.70	1.54
LP+M3	25.44	6.01	25.93	22.17	45.89	21.78	1.13	0.73	1.91

Notes: ME is calculated value, other nutrient levels are measured values based on dry matter. HP: 32% CP (high protein level diet); LP is 24% CP (low protein level diet); LP+M1 is 24% CP+0.3% Met; LP+M2 is 24% CP+0.6% Met; LP+M3 is 24% CP+0.9% Met.

N-balance experiments

The N-balance experiments were carried out with nine male minks from the respective treatment groups. The collection period of feces and urine is lasted three days (October 25−27, 2009). The animals were kept in metabolic cages for separating collection of feces and urine (Jørgensen et al. 1973). Feces and urine were collected quantitatively daily and stored at −20 °C until analyzed. To avoid ammonia evaporation from the urine, 20-mL sulphuric acid (5% solution) was added to the bottles of urine collected, and the urine collection trays were sprayed with citric acid (20% solution) once per day. In the N-balance calculations, the retained N was determined as ingested N- (fecal N+urinary N).

Chemical analyses

The chemical composition of diets and feces was analyzed by standard methods. The contents of dry matter (DM), ash and crude protein (CP: Kjeldahl-N×6.25), calcium, and phosphorus were analyzed according to AOAC (1990) procedures. Crude carbohydrate (CC) was calculated by subtracting ash, CP and ether extract (EE) from the DM content. The calculation of metabolizable energy (ME) content and the proportional composition of ME were based on the digestibility coefficients and the ME values of nutrients were as follows: protein 18.8$MJ \cdot kg^{-1}$, fat 39.8 $MJ \cdot kg^{-1}$, and carbohydrate 17.6 $MJ \cdot kg^{-1}$ (Hansen et al. 1991). The concentrations of amino acids were determinate by Agilent 1100 High Performance Liquid Chromatographic (Agilent Technologies, Inc. Santa Clara. USA). All chromatographic procedures were performed at room temperature, and the samples and standards were evaluated in duplicate (Sedgwick et al. 1991). Serum urea nitrogen concentration and total serum protein content were measured according to the method of Bardford (1976) using kit (Nanjing Jiancheng Biotechnology Co., Ltd, Jiangsu, China) as the standard.

Statistical analyses

All data were analyzed using the GLM procedure of SAS appropriate for a randomized complete block design (SAS 2002). A level of $p<0.05$ was set as the criterion for statistical significance. Dates were represented as (mean±s.d).

Results

Performance and nutrient digestibility

Effects of low-protein dietary with supplementing Met on performance and nutrient digestibility were shown in Table 3. Initial weights, final weights and average daily feed intake (ADFI) of minks were similar in all groups ($p > 0.05$), but final weight of treatment LP+M2 was higher than that of other groups. Apparent digestibility of DM and EE was not impaired with different dietary protein levels ($p > 0.05$). Apparent digestibility of CP was effected by different dietary protein levels ($p < 0.05$) to be decreased with declining dietary protein level, but there was not significant difference between HP and LP+M2 ($p > 0.05$).

N-balance

Contents of N intake and Urinary N decreased with declining dietary protein levels ($p < 0.05$), (Table 4). In treatment HP, contents of N intake and Urinary N were higher than those of other groups ($p > 0.05$). The content of fecal N was not affected by different diets ($p > 0.05$). Daily retention of N (g per mink) was significantly affected by different diets ($p < 0.05$). N retained of treatment LP+M2 was the highest one, but N retained of LP was the lowest one. Contents of N retained were similar among all treatments except of treatment LP, which are 0.96 $g{\cdot}d^{-1}$, 0.77g/d, 0.97 $g{\cdot}d^{-1}$, 1.12 $g{\cdot}d^{-1}$ and 1.09 $g{\cdot}d^{-1}$. Levels of SUN and TP in minks' blood were not affected by different dietary protein levels ($p > 0.05$).

Table 3. Effects of low-protein dietary supplemented with Met on performance and nutrient digestibility of minks

Treatments	Initial weights(g)	Final weights(kg)	ADFI(g)	Digestibility of DM (%)	Digestibility of CP (%)	Digestibility of EE (%)
HP	1.94±0.21	1.99±0.36	93.76±11.37	82.96±1.95	76.69±1.21^{a}	94.71±2.79
LP	1.92±0.19	2.04±0.17	96.94±9.10	82.60±3.36	71.42±1.14^{b}	94.26±1.19
LP+M1	2.06±0.10	2.19±0.24	96.87±17.26	82.04±1.53	72.07±1.11^{b}	92.33±2.29
LP+M2	2.07±0.28	2.23±0.32	94.97±14.38	81.36±1.64	76.19±2.01^{a}	92.34±2.71
LP+M3	2.04±0.21	1.99±0.24	99.98±14.31	81.58±1.67	72.35±1.96^{b}	93.36±2.26
P Value	0.1870	0.1228	0.2343	0.5875	0.0421	0.1775

Notes: The values are the mean ± s.d.; Values within rows with different letters differ significantly ($p < 0.05$). HP is 32% CP (high protein level diet); LP is 24% CP (low protein level diet); LP+M1 is 24%CP+0.3%Met; LP+M2 is 24% CP+0.6% Met; LP+M3 is 24% CP+0.9% Met.

Table 4. Effects of low-protein diets supplemented with Met on N-balance of minks

Treatments	N intake ($g{\cdot}d^{-1}$)	Fecal N($g{\cdot}d^{-1}$)	Urinary N($g{\cdot}d^{-1}$)	N retained($g{\cdot}d^{-1}$)	SUN($mg{\cdot}mL^{-1}$)	TP ($mg{\cdot}mL^{-1}$)
HP	4.90±0.59^{a}	1.21±0.29	2.84±0.13^{a}	0.96±0.18^{ab}	228.90±11.18	75.46±13.49
LP	3.87±0.36^{b}	1.14±0.17	1.92±0.33^{b}	0.77±0.09^{c}	218.25±25.38	84.00±8.73
LP+M1	3.91±0.69^{b}	1.10±0.24	1.87±0.16^{b}	0.97±0.11^{ab}	218.52±29.01	86.66±7.18
LP+M2	3.92±0.59^{b}	1.05±0.09	1.77±0.24^{c}	1.12±0.08^{a}	218.76±36.36	89.91±6.94
LP+M3	4.15±0.59^{b}	1.15±0.22	1.91±0.21^{b}	1.09±0.16^{a}	219.33±51.16	85.99±14.16
P Value	0.0017	0.2139	0.1978	0.0128	0.0643	0.0517

Notes: The values are the mean ± s.d.; Values within rows with different letters differ significantly ($p < 0.05$). HP is 32% CP (high protein level diet); LP is 24% CP (low protein level diet); LP+M1 is 24% CP+0.3% Met; LP+M2 is 24% CP+0.6% Met; LP+M3 is 24% CP+0.9% Met.

Skin characteristics

Effects of low-protein dietary supplemented with Met on skin characteristics were shown in Table 5. No significantly difference was in body length among all experimental treatments ($p>0.05$). However, skin length was significantly affected by different diets ($p<0.05$). Skin length of treatment LP+M2 was a little higher than that of HP. The length of skin in treatment LP+M2 and HP was higher than that of all other groups. The length of guard hair and under hair was not affected by different protein levels ($p>0.05$).

Discussion

Performance and nutrient digestibility

High protein level did not improve the weight gain of minks during the growing-furring period. Our study showed that weights of minks were similar among all groups. After

mid-September, muscles and other tissues were primarily formed in the early growth phase of mink kits. During growing-furring period, dietary protein was mainly supplied for pelt development of minks (Berg 1986). Our research showed that the digestibility of CP was decreased with declining dietary protein level. There are many studies on the influence of protein level on the apparent digestibility of protein in minks (Skrede 1976). Research on ileal-fistulated of blue foxes showed that there is a slight reduction in the apparent digestibility of CP at lower protein levels (Szymeczko et al. 1991). Similar results were obtained from study on mink (Skrede 1979). The influence of protein level on the apparent digestibility of protein is shown under intensive research in other animals, pigs in particular. A number of studies suggest that reductions in dietary protein lead to increases in relative amount of endogenous N secretion, which, in turn, reduce the apparent digestibility of protein (Low 1980). In contrast, Li et al. (1993) showed that the apparent digestibility of protein is not affected by the dietary level of protein. The results indicated that low-protein diets supplemented with Met do not affect the growth performance of minks in growing-furring period.

Table 5. Effects of low-protein diets supplemented with Met on skin characteristics of minks

Treatments	Body length (cm)	Skin length (cm)	Body weight (kg)	Skin weight (kg)	Guard hair length (cm)	Under hair length (cm)
HP	45.14±2.19	76.62±2.97^{ab}	1.99±0.36	215.25±29.00	2.16±0.09	1.44±0.12
LP	44.75±1.28	74.37±1.84^{bc}	2.04±0.17	195.50±18.18	2.10±0.18	1.36±0.11
LP+M1	44.37±0.91	74.37±1.56^{bc}	2.19±0.24	195.62±15.94	2.14±0.20	1.41±0.20
LP+M2	44.50±0.75	77.12±2.23^{a}	2.23±0.32	206.37±18.57	2.20±0.15	1.44±0.09
LP+M3	44.75±0.88	72.38±2.06^{c}	1.99±0.24	199.50±35.20	2.05±0.20	1.25±0.05
P Value	0.8123	0.0007	0.1228	0.4526	0.4755	0.0823

Notes: The values are the mean ± s.d.; Values within rows with different letters differ significantly ($p < 0.05$). HP is 32% CP (high protein level diet); LP is 24% CP (low protein level diet); LP+M1 is 24% CP+0.3%Met; LP+M2 is 24% CP+0.6% Met; LP+M3 is 24% CP+0.9% Met.

N-balance

Animals had less protein intake with reducing dietary protein level. Early study showed that the lower the protein level in the diet the better the utilization of N and the smaller the proportion excreted (Dahlman 2003). In the present investigations, contents of N intake and urinary N declined with reducing dietary protein level, but they did not decline with Met adding in low-protein diet. Glem-Hansen (1980) pointed out that the growing minks fed with low-protein diets were able to compensate for previous low nitrogen retention during the growing-furring period. From an environmental point of view, a substantial reduction in urinary N and fecal N emissions can be achieved by decreasing the dietary protein level with supplementing Met. According to the present research, N excretion in urine declined significantly when the protein level in the diet was lowered from 32% to 24% with supplementing Met, and 24% CP with supplementing Met did not affect the performance of minks, resulting in a decrease of 37.9% in urinary N excretion in growing-furring minks. The requirement for sulfur amino acid is in line with effect of developing hair, fur quality and also growth (Børsting et al. 1996; Dahlman et al. 2002). Our results indicated that mink needs enough Met, but supplementation of Met had a prime quantity. Our results showed that 1.54% Met in diet was enough for minks in growing-furring period.

SUN was affected by dietary protein levels, which was reduced by declining CP level in the present study. Same results had been found in other reports (Kerr et al 1995; Figueroa et al. 2002; Nyachoti et al. 2006; Yue et al. 2008). No significant difference was found in total serum protein among all groups. The results are consistent with earlier findings. Blome et al. (2003) reported that TP concentration in plasma was not significantly different in pigs with different content of dietary protein.

Skin characteristics

In our study, dietary protein level affected the skin characteristics of minks. The best fur quality was achieved in the high protein level of treatment (LP+M2). During the fur growing period, the minks need protein for pelt development (Berg 1986). According to previous reports of effects of dietary amino acids on fur quality of blue fox (Työppönen et al. 1987), low protein content (ME from protein 22%–18%) had only slightly negative effect on fur characteristics. Furthermore, additional Met did not improve fur quality. In recent research on mink, protein level of 30% ME was found to support normal growth performance (Damgaard et al. 1998). A supplement of essential amino acids in diets had a positive effect on growth performance of mink, compared with non-supplemented low protein diets (15%–20% ME). In our study, declined level of dietary protein with supplementing prime quantity of Met could increase the skin length. The body length did not affect the skin length, because skin length may be affected by variations in processing from skinning to drying. Skin length had a same trend with the final body weight of minks, the skin size is positively correlated with body weight rather than with body size, the result was similar as the previous study (Dahlman 2003).

Conclusions

Based on the results from this experiment, addition of Met in low-protein diets for minks could reduce the N emission without

affecting the normal growth. Dietary protein was lowered from 32% to 24% with supplementing Met, resulting in a 37.9% decrease in urinary N excretion in growing-furring minks. SUN level was affected by dietary protein and Met levels. Total serum protein level of minks was irrespective of dietary protein and Met levels. Our results indicated that 24% CP diet supplemented with 1.54 % Met did not affect the skin characteristics of minks in this period. Furthermore, low protein diets added of Met for minks would be beneficial in terms of reducing feed expenses and decreasing nitrogen emissions to the environment.

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